SYNOPSIS
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BIOSYNTHESIS OF NANOPARTICLES FROM MICROBES

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SYNOPSIS

Microbes are the microscopic organisms which are single or multicellular found in all universes. They include the bacteria, algae, fungi and protozoa. Microbes play an important role in balancing the ecosystem by various processes like biodegradation, sewage treatment, soil fertility and improving agricultural productivity. Microbes are known as decomposer in the ecosystem especially fungi well it grows on living or on dead organism.

A microbe produces several hydrolytic enzymes like Amylases, proteases, Lactase, Pectinases, Catalase, Penicillinase, Glucosidases etc. Mycotoxins like Aflatoxin, Zearalenone, Ochratoxin, Citrinin, T-2 toxin, Fumonisins etc. Pigments like qunonine, phynolic group, and also synthesizes the Nanoparticles like Silver(Ag), Gold(Au), Platinum(Pt), Copper(Cu) (Ahmed et al., 2002; Ahmed et al.,2003; Marcato et al.,2003; Bansal et al .,2004).

Nanoparticles are the ultrafine particles of the metals sized between 1 to 100nm. Original properties of the materials can change after the formation of Nanoparticles. Nanoparticles have superior properties than bulk materials. They are more reactive than the larger particles due to their greater surface area per weight. Nanoparticles are more stable for long period. The word ‘Nanoparticles’ comes from the nanotechnology.

Nanotechnology is the science and technology of small things i.e. nano this term originate from Greek word meaning ‘dwarf’. This term was firstly used by ‘Richard Feynman’ in 1959. Therefore nanotechnology term was used from last 53 years. Nanotechnology is multidisciplinary branch combines physics, chemistry, biology, electrical engineering, biophysics, material science etc. Due to their small size, unique properties, control the structure and composition on the nanometer scale

nanotechnology have very much importance. Nanotechnology is the new succeeding tool for medical and biological field develops by nanotechnology. It combines biological principles with physical and chemical procedure to generate nano sized particles with specific function. last few years nanoparticles produced by physical and chemical method for the production of large quantities of nanoparticles which are complicated, outdated, expansive, and produce hazardous toxic wastage that are harmful to environment as well as human health also.

To solving this problem and large quantity nanoparticles production the new method arises called as biological method. This is proper alternative to physical and chemical method because it is nontoxic, eco-friendly, recent, modern and safe (Mohanpuria et al., 2008; Rai et al., 2008; Sharma et al., 2009). In biological method nanoparticles synthesis was carried out without addition of any reducing agent and the stabilizer which are replaced by molecules produced by living organism i. e. bacteria fungi, yeast, algae, plant etc. therefore these living organism are the nanofactories for production of nanoparticles.

Bacteria, fungi, yeast, algae, actinomycetes synthesis silver (Ag), gold (Au), cadmium sulphate (Cds), zinc sulphate (Zns), platinum (Pt), and palladium (Pd) nanoparticles. Out of microbes fungi are most effective good candidate in synthesis of metal Nanoparticles and large scale production, Because fungi secrete large amount of proteins and enzymes for reducing the metal ion and increasing productivity , large amount of biomass production and fungi have very high wall binding capacity. Nanoparticles synthesis from fungi known as Myconanotechnology which has great demand .Due to their monodispersity today fungi are bionanofactories if various metal nanoparticles like silver(Ag), gold(Au), platinum(Pt), Cds. Nanoparticles production source is intracellular or extracellular which depend on the reduction enzyme present in it.

Nanoparticles have multifunctional used in various branches. Silver, gold nanoparticles have great antimicrobial property, which are used in various medicines for detection of diseases. Nanoparticles also used in fabric, in manufacturing materials, electronic devices, solving environmental problem etc. therefore biological synthesized Nanoparticles have tremendous amount of application than the chemically synthesized nanoparticles. Thus the use of microorganism to synthesized functional nanoparticles has been of great interest. Micro-organism can change the oxidation state of metals.

These microbial processes have opened up new opportunities for us to explore novel application such type of bottom –up approach has become an attractive focus in current and future generation.

Considering the importance of this fact present topic has become selected for research entitled **"Biosynthesis of Nanoparticles from Microbes"** with following scheme of working for research.

Present research work is carried out in two parts. The first part of research work is mainly carried out on collection, isolation and screening of fungi which synthesize different type of nanoparticles. For this purpose different sources from different habit and habitat is used for collection and screening of fungi. Fungi were isolated from different soil sample by serial dilution method , deteriorated plant part were collected from different fields, garden, research center etc. which were further employed for isolation of fungi by standard technique.

Isolation of fungi from different source was scientifically categorized and after identification of scientific manuals pure culture was maintained on PDA slants for further study. In all 35genera 78 species were scientifically identified and further screen for the study of biosynthesis of nanoparticles by using different fungal

growth media potato dextrose agar (PDA), Glucose nitrate agar (GNA), Rose Bengal agar (RBA) and Czapek dox agar (CZA). It is observed that potato dextrose agar (PDA) media is found to more favorable for growth of fungi and biosynthesis of nanoparticles.

On the basis of vividly different incidence variation of fungi only 12 fungi belongs to 7 genera were selected for further detailed study of nanoparticles. Selected fungi are as ***Alternaria altrenata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium oxysporum, Helminthosporium tetramera, Macrophomina phaseolina, Penicillium sp., Penicillium chrysogenum, Trichoderm asperllum, Trichoderm harzianum, Trichoderm viride.***

For comparative study of growth pattern, production of pigments ,enzyme, proteins the four synthetic liquid media like Glucose nitrate broth (GNB), Rose Bengal broth (RBB), Czapek dox broth (CZB) and MGYP broth (Malt, Glucose, Yeast, Peptone) were selected. Fungi were alone to grow in control laboratory growth for 6 day. Out of four broth media Glucose nitrate broth (GNB) shows large growth and pigment production than MGYP broth, but Rose Bengal broth (RBB), Czapek dox broth (CZB) shows very little growth hence pigment production was also less. Therefore Glucose nitrate broth (GNB) media is found to be more favorable for growth and synthesis of nanoparticles than other media which are used for further study.

Effect of physical factor such as wavelength, pH, temperature, light and different time interval also study for quantitive and qualitative analysis of nanoparticles biosynthesis. UV-Spectrophotometer reading was taken in between 200-900nm but nanoparticles peck formation was observed in 300-600nm. Therefore 300-6000nm wavelength was selected for further experiment. Effect of different pH factor was also done. At 6.5 pH nanoparticle synthesis and optimum value for maximum

synthesis was found to be compared to other levels (3.5, 4.5, 5.5, 7.5, and 8.5). Highest absorbance was seen at this pH. At different time interval (12, 24, 36 hours) color intensity of nanoparticles becomes increases i.e. large amount nanoparticles formation occurred.

In the second part of work experiment will be mainly carried on production of nanoparticles. GNB media was selected for the production of biomass of 12 fungi. After six days crude cell filtrate and metal salt solution was used for production of silver, gold and copper nanoparticles. The metal salt like silver nitrate ($AgNo_3$), Chloroauric acid ($HAuCl_4$) and Copper sulphate ($CuSo_4$) was used for the production of silver (Ag), Gold (Au), and copper (Cu) nanoparticles. After addition of 1mM solution of $AgNo_3$, $HAuCl_4$ and $CuSo_4$ in to the crude cell filtrate. The colour of crude cell filtrate becomes changes after time interval.

In preliminary confirmation when color of crude cell filtrate become brown then presence of silver nanoparticles, When purple color appeared then gold nanoparticles are present and when yellow color appeared then copper nanoparticles are present. Photometric observation can be down by UV-Spectrophotometer. 300-600 nm wavelengths can be selected .Silver nanoparticles peck formation was observed at 400-550 nm. Out of 12 fungi six fungi shows peck formation at 450 nm, six fungi shows pick formation at 400 nm. Large amount of silver nanoparticles synthesis was occurred in ***Fusarium oxysporum*** while very less amount in ***Trichoderm viride.***

Gold nanoparticles peck formation was observed at 550 nm. Out of 12 fungi only eight fungi shows peck formation at 550 nm. ***Alternaria altrenata, Aspergillus flavus, Penicillium spp., Trichoderm asperllum*** does not showed synthesis of gold

nanoparticles. ***Fusarium oxysporum*** shows large amount of gold nanoparticles while very less amount in ***Aspergillus fumigatus.***

Peck formation of copper nanoparticles synthesis can be occurred at 550-600 nm. All 12 fungi give positive response. At 550 nm the 10 fungi shows peck while two fungi ***Aspergillus flavus*** and ***Aspergillus niger*** shows peck at 600 nm. Large amount of copper nanoparticles observed in ***Trichoderm asperllum*** while very less amount in ***Aspergillus niger.***

Detection of type of synthesis in silver, gold and copper nanoparticles was also done. Out of 12 fungi ***Aspergillus niger*** and ***Fusarium oxysporum*** shows intracellular and extracellular synthesis of silver nanoparticles, while 6 fungi shows only extracellular and four fungi shows intracellular synthesis of silver nanoparticles. In gold nanoparticles synthesis ***Fusarium oxysporum*** synthesized intracellularlly and extracellularlly while four fungi shows extracellularlly and three fungi shows intracellular synthesis.

For the detailed structure i.e. size and shape of nanoparticles Transmission Electron Microscopy (TEM)) technique was used. TEM technique is the confirmationary test of nanoparticles. The characterization of 12 fungi synthesized AgNPs and 5 fungi synthesized AuNPs was down by TEM Morgagni 268D. Out of 12 fungi both silver and gold nanoparticles were synthesized in 5 fungi they are ***Aspergillus niger, Fusarium oxysporum, Helminthosporium tetramera, Macrophomina phaseolina*** and ***Trichoderm harzianum.*** Nanoparticles size was found in between 1-50nm. Fungus synthesized AgNPs are polydisperse spherical, some are ellipsoid in shape occasionally triangular in shape, while as AuNPs are non-uniform triangular, spherical and hexagonal in shape. The smallest average size of silver nanoparticles was found in ***Fusarium oxysporum*** is 10.18nm while large average size was found

in ***Aspergillus fumigatus*** is 41.55nm. ***Helminthosporium tetramera*** synthesized the smallest gold nanoparticles with the average size of 11.24 nm while as ***Aspergillus niger*** synthesized the largest AuNPs with the average size of 68.19 nm.

A biological property of some nanoparticles isolates from fungi is show in differently kind in antimicrobial properties. Antimicrobial analysis is the most important technique due to the antibiotic resistance of microorganism. Antibacterial activity of fungus synthesized silver, gold and copper nanoparticles were checked. Silver nanoparticles were observed great antimicrobial effect on ***Bacillus subtilis, E. coil, K. pneumoniae, P. aeruginosa, Salmonella*** **sps,** ***Shigella*** **sps,** ***Streptococcus sp.*** Agar well diffusion technique was used for the investigation.

Antibacterial effects of silver nanoparticles were observed against human pathogen comparison with the standard drug streptomycin. Overall antibacterial experiment was indicating that the silver nanoparticles have considerable antibacterial activity comparison with the standard antimicrobial drug. The zone of inhibition was observed in both gram positive and gram negative bacterial culture. Therefore these silver nanoparticles have deserving further investigation for clinical application.

In the last attempts biosynthesized nanoparticles shows effect on seed germination. For this experiment seeds of Jawar, Safflower, Soybean, and Sunflower were used. In which we observed that the effect of silver and gold nanoparticles solution on the percentage of seed germination and root shoot length. Due to the silver and gold nanoparticles solution the percentage of seed germination was higher than the control while as the root-shoot length of the seed was smaller than the control.

Therefore this result was indicate that the silver and gold nanoparticles are effective for the seed viability and breaking the seed dormancy for seed germination which are useful for the farmer to increases the production of yield .

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